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## Biglycan (BGN)

Biglycan (BGN) is a small, generally cell surface or pericellular proteoglycan composed of a ~38 kDa core protein modified with several *N*-linked oligosaccharides and, as its name implies, two chondroitin (bone) or dermatan (most soft tissues) sulphate glycosaminoglycan chains. It is member of a large superfamily of proteins that contain various number of tandem, ~25 amino acid repeats characterized by ordered spacing of hydrophobic amino acids, particularly leucine. The small, leucine-rich proteoglycans make up a discrete subfamily characterized by two highly conserved cysteine loops that flank the tandem repeats. Many functions have recently been proposed for biglycan, but its apparent ability to bind members of the TGF- $\beta$  superfamily is one most often cited.

### ■ Synonymous names

Biglycan has several synonymous names generally reflecting its relative electrophoretic position on SDS-PAGE or time of elution from various purification columns. The names include PG-1, PG-I, DS-PGI, PG-S1, and DS-I.

### ■ Protein properties

Biglycan is a member of a growing family of small proteoglycans whose unifying characteristics are two highly conserved cysteine loops flanking 5-10 tandem repeats with BGN having 10.<sup>1</sup> Each repeat is nominally ~25 amino acids in length and is based on the pattern LxxLxLxxNxLx<sub>(12-14)</sub>. For BGN, the two glycosaminoglycan (GAG) chains are chondroitin sulphate in bone matrix and dermatan sulphate in most soft tissues. Other members of this family include decorin (DCN), fibromodulin, lumican, epiphygan, keratocan, and PG-LB (known as DSPG3 in human) (for a review see ref. 2). The biglycan sequence from a number of mammalian species has been reported, including human,<sup>1</sup> cow,<sup>3,4</sup> mouse,<sup>5</sup> and rat.<sup>6</sup>

Curiously, no avian sequences have yet been reported for this highly conserved proteoglycan. Using human BGN as the model, biglycan has 368 amino acids (~41 700 Da) including 19 in the leader sequence and 18 more in the amino terminus that are often removed and are therefore considered to be a propeptide region.<sup>1</sup> Because the propeptide appears to remain on the proteoglycan in tissues such as the epidermis where little or no extracellular matrix accumulates, our working hypothesis is that the prepeptide may be used to bring the BGN to the cell surface and to hold it in place. According to this hypothesis, the propeptide is removed whenever the BGN is released into the surrounding matrix. In tissues such as cartilage and bone, the propeptide is generally removed and the proteoglycan is found in the extracellular matrix. The 'mature' core protein (lacking the prepeptide), made by removing the disaccharide repeats of the GAG chains with chondroitinase ABC, is typically a single band of *M<sub>r</sub>* ~45 000 on SDS-PAGE.<sup>7</sup> This core protein contains the two GAG chain linkage regions (on amino acids 42 and 47 as numbered with the starting Met=1), two *N*-linked oligosaccharides (amino acids 270 and 311) and possibly one or more *O*-linked oligosaccharide chains at unknown locations.<sup>1</sup>

The human biglycan gene was mapped to the X chromosome at Xq27-ter<sup>1</sup> and then to within 700 kb of the DX552 marker.<sup>8</sup> The mouse gene, *Bgn*, has been mapped to X29.3, approximately 50 kb distal to *DXPas8*.<sup>9</sup> Both the human<sup>10</sup> and the mouse<sup>11</sup> genes contain dinucleotide repeats that may be useful for linkage analyses.<sup>12,13</sup> The ~8 kb human gene has been cloned within a single Lambda Fix phage and consists of 8 exons, the first of which does not contain any coding sequences.<sup>10</sup> The organization of the BGN gene is identical to that of the human decorin (DCN) gene<sup>14</sup> strongly suggesting that they are the direct result of gene duplication followed by divergent evolution. The human BGN gene is subject to X-inactivation but it is transcribed like an X-Y homologous gene, suggesting that transacting elements control-



**Figure 1.** Immunolocalization of human biglycan in a hand of a 15-week fetus using antiserum LF-15. BGN is located in the epidermis, vasculature, type I collagen-containing bone, and in the very cellular articular ends of each bone rudiment.<sup>16</sup> This figure should be contrasted with a serial section stained for the related proteoglycan, decorin, on p. 409. Notice that the two are often mutually exclusive in their distribution. (See ref.16 for more details.)

ling the expression of the gene are on both the X and Y chromosomes.<sup>15</sup>

Biglycan has been localized to a number of tissues, often to areas distinctly different from that of decorin. In fetal humans, BGN has been observed by both immunohistochemistry and *in situ* experiments in kidney endothelia and collecting tubules, endocardium, and some myocardial fibres in the heart, endothelia, intima and media of the aorta, the epidermis of the skin, but generally not the dermis (except in endothelial cells of the vasculature), myofibre of skeletal muscles and all mineralized bone rudiments. In the developing cartilage of the skeleton, the BGN was seen most strongly in the highly cellular areas that give rise to chondrocytes, the growing 'caps' in articular cartilage of the appendicular elements and the inner portions of the vertebral rudiments.<sup>16</sup>

### ■ Purification

Chondroitin sulphate-containing BGN can be purified from fetal or young bone by a series of extraction proce-

dures and protein chromatography.<sup>7</sup> Bone is milled into a fine powder, extracted with denaturing buffers to remove blood and cellular proteins, and the residue extracted with demineralizing buffer. Standard molecular sieve and ion exchange chromatography in denaturing buffers are performed. In our hands, reverse phase chromatography using standard organic solvents results in large losses. Dermatan sulphate-containing BGN can be isolated in good yield from articular cartilage using similar procedures as well as a reverse phase column and a detergent gradient.<sup>17</sup> We have had little luck generating soluble recombinant BGN in *E. coli* but a vaccinia-based recombinant method has been reported using UMR106 and HT-1080 as host cells resulting in ~10 mg of BGN per billion cells per day.<sup>18</sup>

### ■ Activities

While the function of BGN has not been unambiguously assigned, several studies have been published that suggest a wide range of possible functions. BGN has been shown to bind to type I<sup>19</sup> and type V<sup>18</sup> collagen, TGF- $\beta$ ,<sup>20</sup> and the complement protein C1q.<sup>18</sup> BGN has been shown selectively to increase interleukin 7-dependent proliferation of pre-B cells<sup>21</sup> as well as increase the survival of brain neocortical neurones *in vitro*.<sup>22</sup> Localization of this proteoglycan to the tips and edges of the lamellipodia of migrating endothelial cells has suggested that it may be involved in the control of cell migration.<sup>23</sup>

### ■ Antibodies

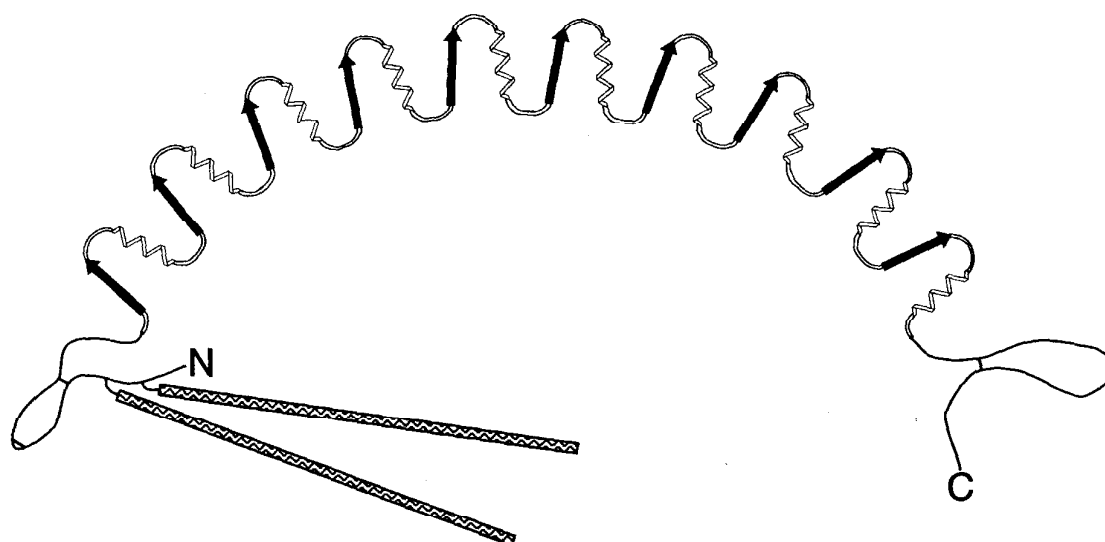
No monoclonal antibodies for biglycan are currently listed in ATCC's Hybridoma Data Bank (<http://www.atcc.org/hdb/hdb.html>). Limited amounts of the following rabbit (polyclonal) antisera are available to colleagues for research purposes only. Any use must comply completely with local and NIH's guidelines for patient care and confidentiality.

### ■ Genes

Full length BGN cDNA for human (clone P16, GenBank accession number J04599)<sup>1</sup> and mouse (clone 3, GenBank accession number L20276) are available from our laboratory for experimental use only. The human BGN gene in Lambda Fix DNA is also available in small quantities. Any use must comply completely with local and NIH's guidelines for patient care and confidentiality.

### ■ Mutant phenotype/disease states

No known disease has been attributed to changes in BGN. Dr Marian Young's laboratory at the National Institute of Dental Research, NIH has successfully produced a viable BGN knockout mouse, but its phenotype has not yet been published.



**Figure 2.** Diagram of the structure of biglycan based loosely on the bent-coil structure determined for the leucine-rich repeat structure of the porcine ribonuclease inhibitor.<sup>24</sup> The arrows represent short  $\beta$  sheets and the two straight rods near the N terminus represent the two glycosaminoglycans that give biglycan its name. BGN is thought to contain three disulphide bonds, represented by short connecting lines. (Drawn by Dr Andrew Hinck, NIDR, NIH.)

**Table 1**

| Gene product         | Antiserum | Antigen                        | Known species   |
|----------------------|-----------|--------------------------------|-----------------|
| Human BGN            | LF-15     | GVLDPDSVTPTYSA-(BSA)           | H, M            |
| Human BGN            | LF-51     | GVLDPDSVTPTYSA-(BSA)           | H, M            |
| Human BGN            | LF-112    | GVLDPDSVTPTYSA-(BSA)           | H, M            |
| Human BGN            | LF-121    | Recombinant BGN (w/propeptide) | H, M            |
| Human BGN propeptide | LF-104    | LPFEQRGFWDFTLDDC-(LPH)         | H, M, R, Mou    |
| Human BGN propeptide | LF-105    | LPFEQRGFWDFTLDDC-(CSA)         | Same as LF-104? |
| Bovine BGN           | LF-96     | LPDLDSLPTYSC-(LPH)             | Only cow tested |
| Bovine BGN           | LF-97     | LPDLDSLPTYSC-(CSA)             | Only cow tested |
| Mouse BGN            | LP-106    | VPDLDSVTPTFSAMC-(LPH)          | R, Mou tested   |
| Mouse BGN            | LP-107    | VPDLDSVTPTFSAMC-(CSA)          | R, Mou tested   |

All antisera are whole rabbit sera.

H, human; M, monkey; R, rat; Mou, mouse; LPH, horseshow crab haemocyanin; CSA, chicken serum albumin.

## Structure

The three-dimensional structure of BGN has not been determined. By analogy study on a porcine ribonuclease inhibitor (another protein with similar leucine-rich repeats),<sup>24</sup> we expect the structure of BGN to be dominated by bent-coil structure. In this hypothetical structure, each of the 10 repeats forms a single turn of the coil with each turn slightly angled to produce a structure that is somewhat horseshoe-like in appearance. Unfortunately, the ribonuclease inhibitor does not use the conserved cysteine clusters found in BGN and many other leucine-rich repeat proteins, so we can not infer the structure of the BGN protein outside of the central repeats.

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## Bone sialoprotein (BSP)

Bone sialoprotein (BSP) is a phosphorylated and sulphated glycoprotein that is associated with most normal and many pathological mineralized matrices. It is a small (~M<sub>r</sub> 75 000 Da) integrin-binding protein that supports cell attachment *in vitro* through both RGD-dependent and RGD-independent mechanisms and has a high affinity for hydroxyapatite. It is a member of a family of acidic, integrin-binding sialoproteins which also includes osteopontin, dentin matrix protein (DMP1), and, perhaps, the dentin sialophosphoprotein (DSPP).

### ■ Synonymous names

BSP is the original name<sup>1</sup> but it was also known for a short time as BSP-II.<sup>2</sup> Care must be taken in the literature because of some confusion between BSP and osteopontin which was known originally as BSP-I,<sup>2</sup> as well as an occasional misnaming of the α2HS glycoprotein (or fetuin) as BSP.<sup>3</sup> Because the mouse genome already had used the *Bsp* locus name for another gene, the gene name for the BSP product is *IBSP* (*Ibsp* for mouse) which stands for integrin-binding sialoprotein.

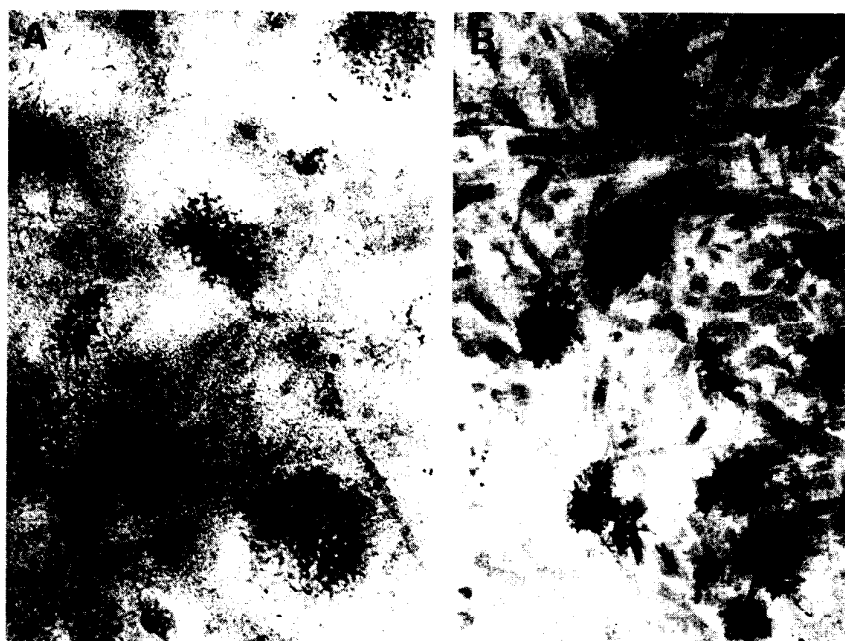
### ■ Protein properties

Bone sialoprotein (BSP) constitutes about 10–15 per cent of the non-collagenous proteins found in the mineralized compartment of young bone.<sup>1</sup> Immunolocalization and *in situ*

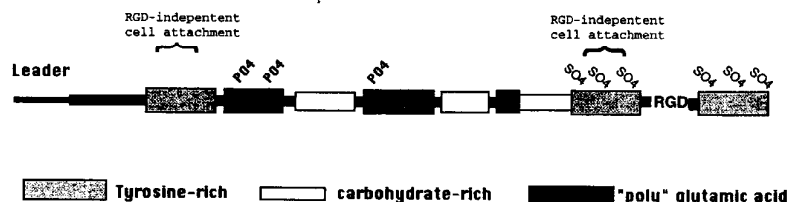
hybridization studies have shown BSP to be made not only by osteoblasts but also by the living cells embedded within bone, the osteocytes, as well as the multinucleated cells that resorb bone, the osteoclasts.<sup>4</sup> The areas richest in BSP are the 'cement lines' or collagen-poor matrix found between areas of new bone, whether that be between the cartilage anlage and bone in development or between old bone and new bone during turnover. Outside of bone, BSP has been found in three other mineralized tissues, dentin,<sup>1</sup> cementum,<sup>5</sup> and calcifying cartilage of the growth plate.<sup>4</sup> BSP expression is usually limited to these skeletal elements. Trophoblasts of the developing placenta, however, express high levels of BSP.<sup>6</sup> While this tissue is not usually considered to be a mineralized tissue, late term human placentas are well known to have hydroxyapatite crystals associated with the ageing trophoblasts.

Recently there have been reports that cancer tumours that are known to form mineralized foci also express BSP. Breast cancer tumours that have a tendency to metastasize to bone often have microcalcifications that show up on mammograms and now have been shown to express high levels of BSP.<sup>6</sup> Indeed, the survival rate of patients with positive lymph nodes but whose tumour biopsies exhibited no expression of BSP was higher than for those patients with BSP-positive tumours but no lymph node involvement.<sup>7</sup> Lung cancers with microcalcifications are also BSP positive and tend to metastasize to bone.<sup>8</sup> Recently two other cancers that have a high propensity to metastasize to bone, prostate<sup>34</sup> and thyroid<sup>35</sup> have also

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**Figure 1.** EM localization of BSP (A) and apatite crystals (B) in similar sections of growing rat bone. Notice the immunogold particles (BSP) associated with the electron-dense matrix between the collagen fibrils. Similar areas fixed to retain the mineral shows the earliest apatite crystals to be located in the same structures. Such stippled, electron dense structures present even at the cell membrane have been shown to contain BSP,<sup>11</sup> suggesting that BSP is present prior to mineral rather than BSP (with its high affinity for apatite,  $2 \times 10^{-9}$  M) simply binding to apatite crystals after mineralization has taken place. Antiserum LF-6 was used in this experiment. See ref. 11 for more details.



**Figure 2.** Diagram showing the various domains of mammalian BSP. Note that there are no disulphide bonds in BSP. The high content of hydrophilic amino acids suggests that the protein is likely to be an extended rod. PO<sub>4</sub> indicate likely phosphorylation sites, others are possible. SO<sub>4</sub> indicate a few of the many tyrosine sulphation sites found flanking the RGD domain. The more amino-terminal tyrosine-rich domain can not be sulphated. Glutamic acid-rich domains and carbohydrate-rich domains are also indicated. RGD represents the integrin-binding tripeptide. Two RGD-independent, tyrosine-rich cell attachment domains are also labelled.

been shown to often express high levels of BSP while their corresponding normal tissues do not.

All of the above localization data plus the fact that this highly acidic protein has a high affinity for apatite<sup>9</sup> leads quite naturally to a hypothesis that BSP may nucleate hydroxyapatite crystals *in vivo*. BSP has been shown to nucleate such crystals *in vitro*.<sup>10</sup> Furthermore, EM localization of BSP in developing bone has shown that electron-dense aggregates that are rich in BSP are secreted into

the matrix and are associated with the earliest mineral crystals.<sup>11</sup> While it seems likely that any cell type that secretes large amounts of BSP over an extended period of time may eventually cause mineral to form (including placentas and tumours) it is not clear that this is the primary function of BSP. The other property of BSP and the other members of the family (osteopontin and DMP1) is the ability to support cell attachment *in vitro* through its integrin-binding tripeptide, RGD. This region has been

shown to be the likely binding site to the vitronectin receptor, the  $\alpha V\beta 3$  integrin.<sup>12</sup> BSP also has two RGD-independent cell attachment domains that have recently been shown to be two tyrosine-rich domains.<sup>9</sup> It is reasonable to hypothesize that cells with BSP bound to their integrins may change their own behaviour (cell shape, ability to migrate, etc.).

Using human BSP as a model<sup>13</sup> the protein is first made as 317 amino acid, 35 000 Da protein. The 16 amino acid leader peptide is removed during synthesis. BSP has no disulphide bonds and it is nearly uniformly hydrophilic along its length suggesting that the protein is likely to be an extended rod in solution. There are three regions particularly rich in glutamic acids residues ('polyglutamic acid domains') that have long been thought to govern the high affinity of this protein for hydroxyapatite. Recent work with recombinant fragments, however, shows that BSP's ability to bind strongly to apatite is found throughout its entire length.<sup>9</sup> Human BSP contains four consensus sequences for N-linked oligosaccharides, three of which are conserved for all mammalian species known to date. These N-linked and the many O-linked oligosaccharides make up approximately 50 per cent of the mass of BSP as it is secreted into the bone matrix.<sup>1</sup> (Curiously, in the rabbit, the BSP is a keratan sulphate proteoglycan.<sup>14</sup>) Tyrosine sulphation and serine/threonine phosphorylation make up the remainder of the known post-translational modifications. There are three tyrosine-rich domains in BSP, the last two of which flank the RGD domain and these two are subject to sulphation. The presence or absence of the sulphate groups did not appear to change the ability of fibroblasts to attach in a simple *in vitro* assay.<sup>15</sup>

The cDNA sequences for rat,<sup>16</sup> human,<sup>13</sup> mouse,<sup>17</sup> cow,<sup>18</sup> hamster,<sup>19</sup> and chicken<sup>20</sup> BSP have been published. The human<sup>21</sup> and chicken<sup>22</sup> genes have also been published. The human *IBSP* gene maps very close to two other members of this family, within 340 kb of *SPP1* (osteopontin) and within 150 kb of *DMP1* with the order being *cen-DMP1-IBSP-SPP1-tel* on chromosome 4.<sup>23,24</sup> Mouse *Ibsp* is on the homologous region of chromosome 5 at 56.0.<sup>17</sup>

## Purification

BSP can easily be purified from developing bone by the use of standard biochemical techniques,<sup>1</sup> although this approach results in protein that has been subject to denaturants. A rat osteosarcoma cell line UMR-106-BSP can produce mg/l-amounts of BSP under serum-free conditions. BSP from the media of these cells can easily be purified to > 95 per cent purity using non-denaturing conditions.<sup>25</sup> The UMR-106-BSP cell line makes what is probably an over-sulphated form of BSP (compared to bone-derived BSP) but the amount of sulphate groups can be lowered to as little as 5 per cent by the use of low sulphate media and chlorate.<sup>15</sup>

## Activities

BSP has no unambiguously assigned *in vivo* activity. It binds with high affinity to hydroxyapatite crystals,<sup>9</sup> collagen, preferably to the  $\alpha_2(I)$  chain<sup>26</sup> and to cell surface receptors including integrins.<sup>17</sup> It can nucleate apatite crystals *in vitro*<sup>10</sup> and probably causes mineralized foci in various pathologies.<sup>4,6,8</sup> BSP has been shown to increase, in a dose-dependent manner, osteoclast resorption in pit assays.<sup>27</sup> It has recently been used as a marker of

- (1) breast cancers more likely to metastasize to bone,<sup>7</sup>
- (2) relative severity of (untreated) multiple myeloma,<sup>28</sup>
- (3) bone turnover, predominantly reflecting resorption aspects,<sup>28</sup> and
- (4) increased joint destruction by determining BSP levels in synovial fluid.<sup>29</sup>

Curiously, *Staphylococcus aureus* cells isolated from patients suffering from bone infections, bound to BSP whereas *S. aureus* from other infections did not bind to BSP.<sup>30</sup> BSP can support cell attachment in an RGD-dependent<sup>12</sup> and RGD-independent<sup>15</sup> manner. The latter property has been determined to reside within the first two of three tyrosine-rich domains.<sup>9</sup> In chicken BSP, the second tyrosine-rich domain has been replaced by two additional RGD domains.<sup>20</sup>

Table 1<sup>33</sup>

| Gene product   | Antiserum    | Antigen                               | Known species      |
|----------------|--------------|---------------------------------------|--------------------|
| Human BSP      | LF-6         | Human bone BSP                        | H, M, D, R, Mou    |
| Human BSP      | LF-83        | YESENGEPGRGDNYRAYED-(LPH)             | H, M, D, R, Mou, C |
| Human BSP      | LF-84        | YESENGEPGRGDNYRAYED-(LPH)             | H, M, D, R, Mou, C |
| Human BSP      | LF-100       | AIQLPKKAGDIC-(LPH)                    | H, M, D, R         |
| Human BSP      | LF-101       | AIQLPKKAGDIC-(CSA)                    | H, M, D, R, P, B   |
| Human BSP      | LF-119       | Recombinant RGD domain (a.a. 257-317) | H, M, D, C         |
| Human BSP      | LF-120       | Recombinant Fragment 1 (a.a. 129-281) | H, M, D, P, R      |
| Human BSP      | LF-125       | Recombinant (a.a. 36-61)              | H, M, P, R, S      |
| Rat BSP        | LF-87        | From UMR-106 media                    | H, R, Mou          |
| <b>Rat BSP</b> | <b>LF-90</b> | <b>From chlorate UMR-106 media</b>    | <b>R tested</b>    |

All antisera are whole rabbit sera.

H, human; M, monkey; D, dog; R, rat; Mou, mouse; C, chicken; P, pig; S, sheep; B, bovine; LPH, horseshoe crab haemocyanin; CSA, chicken serum albumin.

## Antibodies

One monoclonal antibody for rat BSP (1014714) is currently listed in ATCC's Hybridoma Data Bank (<http://www.atcc.org/hdb/hdb.html>). Limited amounts of the following rabbit (polyclonal) antisera are available to colleagues for research purposes only. Any use must comply completely with local and NIH guidelines for patient care and confidentiality.

## Genes

The *IBSP* genes for human<sup>21</sup> and chicken<sup>22</sup> have been reported. The human gene consists of one 5' untranslated exon followed by five small coding exons. The last exon is the largest and contains the integrin-binding RGD domain. Some work on the rat promoter region has been reported.<sup>31</sup> cDNA for human (plasmid B6-5g, GenBank accession number J05213) and mouse (plasmid mBSP1, GenBank accession number L20232) are available from our laboratory for experimental use only. Any use must comply with local and NIH guidelines for patient care and confidentiality.

## Mutant phenotype/disease states

No known disease has been attributed to changes in *IBSP*. The autosomal dominant disorder of dentin formation, dentinogenesis imperfecta type II (DGII), was mapped to the *IBSP* region with no recombination but sequencing of the exons from the patients revealed no disease-specific mutations.<sup>24</sup> A knockout mouse for BSP has been reported in a preliminary study.<sup>32</sup> The mice are smaller, have smaller marrow spaces, smaller secondary ossification sites, and wider articular cartilage.

## Structure

The three-dimensional structure for complete BSP has not been elucidated. The large amount of carbohydrates probably precludes an X-ray diffraction solution and currently at ~75 000 Da, it is too large to solve by NMR. BSP has no disulphide bonds and its high content of hydrophilic and acidic amino acids suggests that it is likely to be an extended structure. The ~60 amino acid, carbohydrate-free carboxy-terminal domain containing the integrin-binding RGD was made rich in <sup>15</sup>N by recombinant technology, analysed by NMR, and found to be a rapidly flexing or random coil.<sup>9</sup>

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## Cartilage matrix protein

Cartilage matrix protein (CMP) is a major non-collagenous protein in the matrix of cartilage of various organs.<sup>1,2</sup> It exists as a disulphide-bonded homotrimer of 148 kDa. In the growth plate during endochondral bone formation, the CMP gene is transcribed specifically by chondrocytes of the zone of maturation. The translation product is distributed in both the zones of maturation and hypertrophy, the two post-mitotic regions of an epiphyseal growth plate. Thus, CMP is a marker for post-mitotic chondrocytes.<sup>3</sup> In primary chondrocyte cultures, CMP forms a filamentous network that consists of both type II collagen dependent<sup>4</sup> and independent filaments.<sup>5</sup> CMP also interacts with aggrecans.<sup>6,7</sup>

### ■ Synonymous names

148 kDa cartilage protein,<sup>2</sup> matrilin-1.<sup>22</sup>

### ■ Homologous proteins

Based on a structure that consists of A domains, EGF-like domains, and C-terminal potential oligomerization domains, matrilin-2 (ref. 22) and matrilin-3 (refs 23 and 24) are homologous to CMP (matrilin-1). Based on the A domains, CMP also has homology to a number of serum, matrix, and cell surface proteins.<sup>8</sup> These are von Willebrand factor, complement factors B and C2, collagen types VI, VII, XII, and XIV, undulin, the  $\alpha$  chains of the integrins VLA-1, VLA-2, LFA-1, Mac-1, P150/95 (in the integrins the A domains are referred to as I domains), and a *Caenorhabditis elegans* protein involved in muscle attachment as well as in malaria thrombospondin-related anonymous protein, dihydropyridine-sensitive calcium channel, and inter- $\alpha$ -trypsin inhibitor.

### ■ Protein properties

CMP exists as a disulphide-bonded homotrimer in the matrix of cartilage. The amino acid sequence of the CMP monomer has been deduced from chicken,<sup>9,10</sup> human,<sup>11</sup> and mouse<sup>12</sup> cDNA and genomic DNA sequences. Each monomer consists of two CMP-A domains which are separated by an EGF-like domain. A heptad repeat-containing<sup>13</sup> tail makes up the C-terminal domain of the protein.

The mature form of a CMP monomer contains 12 cysteine residues. Two of these are in each of the CMP-A domains, six in the EGF-like domain, and two in the heptad repeat-containing tail. A mutational analysis of CMP-trimer formation<sup>14</sup> indicates that the heptad repeats are necessary for the initiation of CMP trimerization and that the two cysteines in the heptad repeat containing tail are both necessary and sufficient to form intermolecular disulphide bonds. The two cysteines within a CMP-A domain form an intra-domain disulphide bond. The heptad repeats are sufficient for the formation of an  $\alpha$ -helical coiled-coil structure<sup>15</sup> and for maintaining the trimeric state of extracted CMP after reduction.<sup>13</sup>

The molecular weight of the intact protein is 148 kDa as determined by sedimentation equilibrium centrifugation.<sup>1</sup> In electrophoretic analyses on SDS-PAGE, CMP migrates as a 200 kDa trimer. Upon reduction of the disulphide bonds, the protein behaves as a single subunit of 54 kDa. In electron microscopic images,<sup>13</sup> CMP is seen as three ellipsoid subunits with approximate values of 7.6 nm for the longer axis and 5.6 nm for the shorter axis. The average diameter of whole molecules is 18 nm.

There is a single copy of the CMP gene in the genome of chicken<sup>9,10</sup> and human.<sup>11</sup> The human CMP gene has been mapped to chromosome 1p35.<sup>11</sup> Both the chicken and human genes consist of eight exons and seven introns. The RNA splice junctions of the seventh intron (intron G) of the chicken and human CMP gene do not conform to consensus splice sequences, suggesting a novel type of splicing mechanism in cartilage. The relationship between the structure of CMP and the CPM gene in the chicken is shown in Fig. 1. A similar organization exists for the human gene. Each of the CMP-A domains is encoded by two exons whereas the EGF-like domain is encoded by a single exon. The exon-intron junction within the CMP-A domains is at a different position within the coding regions of each of the two domains.<sup>10</sup> The exonic composition of the A domains of factor B, p150/95, and vWF show no distinct pattern. This domain in factor B is encoded by five exons and the A3 domain of von Willebrand factor and p150/95 are each encoded by four exons. Domains A1 and A2 of von Willebrand factor are encoded by one single large exon.

In the matrix deposited in primary chondrocyte cultures, CMP interacts with collagen fibrils.<sup>4</sup> The collagen binding of CMP has been localized to the CMP-A domains

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## Decorin

Decorin (DCN) is a small proteoglycan composed of a ~38 kDa core protein usually modified with a single chondroitin sulphate (bone) or dermatan sulphate (most soft tissues) glycosaminoglycan chain and two or three *N*-linked oligosaccharides. DCN is virtually ubiquitous in the matrices of various connective tissues, being found bound to or 'decorating' the collagen fibrils. The protein portion is composed of 10 tandem repeats of ~25 amino acids characteristically rich in ordered leucines with the repeats being flanked by two cysteine disulphide loops. These tandem repeats are found a wide variety of closely related small proteoglycans including: biglycan (BGN), fibromodulin, lumican, epiphykan, keratocan, and PG-Lb. The most commonly cited functions of DCN are its roles in collagen fibril assembly (and stabilization) as well as its ability to bind to TGF- $\beta$ .

### ■ Synonymous names

Decorin has several synonymous names, most reflecting its relative position on SDS-PAGE or time of elution from various purification columns. The names include PG40, PG-2, PG-II, PG-S2, CS-PGII, and DS-PGII.

### ■ Protein properties

Decorin is a member of a growing family of small proteoglycans whose unifying characteristics are two highly conserved cysteine loops flanking 5 to 10 tandem repeats. Each repeat is nominally ~25 amino acids in length and is based on the pattern LxxLxLxxNxLx<sub>(12-14)</sub>. For DCN there are 10 repeats and the single glycosaminoglycan (GAG) chain is chondroitin sulphate in bone matrix and dermatan sulphate in most soft tissues. Other members of this family include biglycan, fibromodulin, lumican, epiphykan, keratocan, and PG-Lb (known as DSPG3 in human) (for a review see ref. 1). The DCN sequences from a number of species have been reported, including human,<sup>2</sup> cow,<sup>3</sup> mouse,<sup>4</sup> rat,<sup>5</sup> rabbit,<sup>6</sup> and chicken.<sup>7</sup> Curiously, the chicken form can have two GAG chains and these chains appear to be attached to a GlySer sites rather than the apparently universal mammalian Ser-Gly.<sup>7</sup> Using human DCN as the model, decorin has 359 amino acids (~39 700 Da) including 17 in the leader sequence and 14 more in the amino terminus that are often removed and are therefore considered to be a propeptide region.<sup>2</sup> The 'mature' core protein (lacking the propeptide), made by removing the disaccharide

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**Figure 1.** Immunolocalization of human decorin in a section of a hand from a 15-week fetus using antiserum LF-30. Dark areas represent the presence of decorin. DCN is localized to all connective tissues, including dermis, cartilage, and type I collagen-containing bone.<sup>10</sup> Compare this with a serial section stained for a closely related proteoglycan, biglycan, on p. 366. Notice that the two are often mutually exclusive in their distribution. See ref. 16 for more details.

repeats of the GAG chain with chondroitinase ABC, is typically a doublet band of  $M_r$  ~45 and 47 kDa on SDS-PAGE.<sup>8</sup> This core protein contains the GAG chain linkage region (on amino acid 34, as numbered with the starting Met as 1) and two or three *N*-linked oligosaccharides on amino acids 211, 262, and 303 (leading to the doublet band.<sup>9</sup>).

The human DCN gene was mapped to human chromosome 12 at either q21.3<sup>10</sup> or q23<sup>11</sup> and location 55.0 on mouse chromosome 10.<sup>12</sup> The human gene has a complex dinucleotide repeat polymorphism that may be useful for genetic studies.<sup>13</sup> The human DCN gene has been cloned within three non-overlapping Lambda Fix clones making the gene at least 25 kb in size.<sup>10</sup> The DCN gene has eight exons with the seven protein-encoding exons matching completely with the homogeneous exons of the human biglycan gene, BGN,<sup>14</sup> strongly suggesting that these two genes were at one time a single gene.<sup>15</sup> Interestingly, DCN has two different, non-translated exon 1 thereby

suggesting that the transcription of this gene is under the control of two promoters.<sup>11</sup>

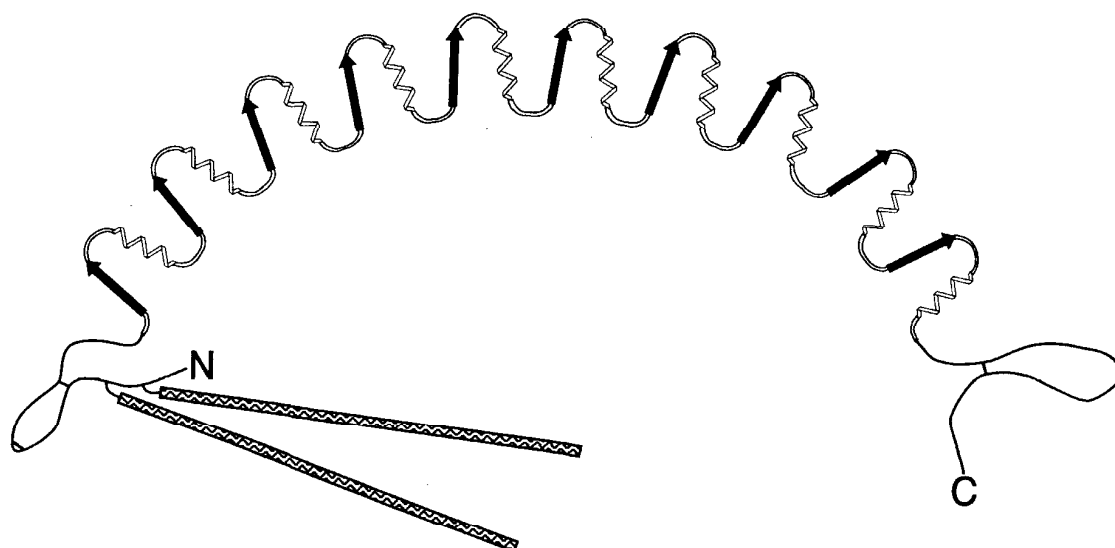
Decorin is found wherever type I, II, or III collagen fibrils are found. This includes not only the major extracellular matrices such as skin and skeleton but also all of the finer support matrices around and within organs of the body.<sup>16</sup> DCN has been localized to the gap regions, near the d and e bands, on the surface of type I collagen fibrils.<sup>17</sup>

## ■ Purification

Chondroitin sulphate-containing DCN can be purified from fetal or young bone by a series of extraction procedures and protein chromatography.<sup>8</sup> Bone is milled into a fine powder, extracted with denaturing buffers to remove blood and cellular proteins, and the residue extracted with demineralizing buffer. Standard molecular sieve and ion exchange chromatography in denaturing buffers are performed. In our hands, reverse phase chromatography using standard organic solvents results in large losses. Dermatan sulphate-containing DCN can be isolated in good yield from articular cartilage using similar procedures as well as a reverse phase column and a detergent gradient.<sup>18</sup> Recombinant DCN with appropriate binding activity has been reported using a maltose-binding fusion protein.<sup>19</sup> For post-translationally modified DCN, a vaccinia-based recombinant method has been reported using UMR106 and HT-1080 as host cells resulting in ~30 mg of DCN per billion cells per day.<sup>20</sup>

## ■ Activities

Decorin has been reported to change the kinetics and final shape of type I collagen fibrils *in vitro*.<sup>21</sup> Indeed, the knockout mouse has fragile skin with unusual collagen fibril morphology.<sup>22</sup> DCN has also been shown to bind to TGF- $\beta$ .<sup>23</sup> This naturally leads to an interesting hypothesis that DCN on the surface of the matrix fibrils may bind TGF- $\beta$  or other members of its superfamily and release these powerful bioactive molecules when the matrix is disrupted in specific ways. Presumably other bioactive proteins may similarly be bound to matrix components. Cells sensing the increase or decrease in the levels of these different active proteins may use such mechanisms to monitor the health of the matrix within its purview. The TGF- $\beta$  binding property has been proposed to be used in protecting against scarring in kidney diseases.<sup>24</sup> Alternatively, another report suggests that decorin induces growth suppression by up-regulating p21, an inhibitor of cyclin-dependent kinases.<sup>25</sup> Decorin also binds to fibronectin and this may explain its propensity to block adhesion *in vitro*.<sup>26</sup> DCN has been reported to induce matrix metalloproteinase collagenase (MMP-1) in synovial fibroblasts adhering to vitronectin. This activity was thought to be independent of the TGF- $\beta$  effects.<sup>27</sup>



**Figure 2.** Diagram of the structure of decorin based loosely on the bent-coil structure determined for the leucine-rich repeat structure of the porcine ribonuclease inhibitor.<sup>24</sup> The arrows represent short  $\beta$  sheets and the straight rod near the N terminus represents the glycosaminoglycan chain. DCN is thought to contain three disulphide bonds, represented by short connecting lines. (Drawn by Dr Andrew Hinck, NIDR, NIH.)

**Table 1**

| Gene product         | Antiserum | Antigen                        | Known species   |
|----------------------|-----------|--------------------------------|-----------------|
| Human DCN            | LF-30     | GIGPEVPDDRDF-(KLH)             | H, M            |
| Human DCN            | LF-136    | GIGPEVPDDRDF-(KLH)             | H, M            |
| Human DCN            | LF-122    | Recombinant DCN (w/propeptide) | H, M            |
| Human DCN Propeptide | LF-110    | QVSWAGPFQQRGLFDC-(LPH)         | Only H tested   |
| Human DCN Propeptide | LF-111    | QVSWAGPFQQRGLFDC-(CSA)         | Only H tested   |
| Bovine DCN           | LF-94     | IGPEEHFPEVPEC (LPH)            | Only cow tested |
| Bovine DCN           | LF-95     | IGPEEHFPEVPEC-(CSA)            | Only cow tested |
| Mouse DCN            | LF-113    | IIPYDPDNPLISMC-(LPH)           | R, Mou tested   |
| Mouse DCN            | LF-114    | IIPYDPDNPLISMC-(CSA)           | R, Mou tested   |

All antisera are whole rabbit sera.

H, human; M, monkey; R, rat; Mou, mouse; LPH, horseshoe crab haemocyanin; CSA, chicken serum albumin; KLH, keyhole limpet haemocyanin.

## Antibodies

No monoclonal antibodies for decorin are currently listed in ATCC's Hybridoma Data Bank (<http://www.atcc.org/hdb/hdb.html>). Limited amounts of the following rabbit (polyclonal) antisera are available to colleagues for research purposes only. Any use must comply completely with local and NIHs guidelines for patient care and confidentiality.

## Mutant phenotype/disease states

There have been no specific human diseases yet unambiguously ascribed to mutations in DCN. There is one report of two osteogenesis imperfecta patients, both

with the same gly-415/ser mutation in the  $\alpha 1(I)$  chain of collagen, in which the patient with the more severe phenotype had little or no decorin production in fibroblasts while fibroblasts from the other patient produced normal amounts of DCN.<sup>28</sup> This suggests that changes in the expression of decorin may have phenotypic consequences in some tissues. The mouse DCN knockout mouse is reported to have fragile skin with coarser and irregular collagen fibrils.<sup>22</sup>

## Structure

The three-dimensional structure of DCN has not been determined. By analogy to the X-ray diffraction study on a porcine ribonuclease inhibitor (another protein with

similar leucine-rich repeats),<sup>29</sup> we expect the structure DCN to be dominated by bent-coil structure. In this hypothetical structure each of the 10 repeats forms a single turn of the coil with each turn slightly angled to produce a structure that is somewhat horseshoe-like in appearance. Unfortunately, the ribonuclease inhibitor does not use the conserved cysteine clusters found in DCN and many other leucine-rich repeat proteins, so we cannot infer the structure of the DCN protein outside of the central repeats.

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## Egg zona pellucida glycoproteins

Mammalian eggs are surrounded by a relatively thick (~2-25 µm) extracellular coat, the zona pellucida (ZP), which consists of three glycoproteins, called ZP1-3.<sup>1-3</sup> These glycoproteins are organized, through non-covalent bonds, into an extensive network of interconnected filaments that exhibit a ~150 Å structural repeat. Free-swimming sperm bind in a relatively species-specific manner to the ZP by recognizing ZP3, the sperm receptor.<sup>4-6</sup> Bound sperm then undergo the acrosome reaction (exocytosis), bind to ZP2, penetrate through the ZP, and fuse with egg plasma membrane (fertilization).<sup>3,7</sup>

Following fertilization, the ZP undergoes structural and functional changes as part of the secondary (slow) block to polyspermy. The various functions of the ZP can be accounted for fully by the properties of ZP1-3 before and after fertilization.

### Protein properties

ZP glycoproteins vary considerably in size among different mammalian species and some of the variability is due

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